

What is claimed is

1. An oligonucleotide that may be used as a primer, in particular a forward primer, to amplify a nucleic acid region of a genital human papilloma virus (HPV) and that has the sequence 5'-CAR GCI AAA WWW KTD AAR GAY
5 TGT G-3', or 5'-CAR GCN AAA WWW KTD AAR GAY TGT G-3' (SEQ ID no. 1), wherein R = A, or G, W = T, or A, K = T, or G, I = inosine, N = A, T, G, or C, D = A, T, or G, and Y = C, or T.
2. The oligonucleotide of Claim 1, wherein the oligonucleotide is selected from the group comprising:
 - 10 a) an oligonucleotide having the nucleotide sequence 5'-CAR GCI AAA TAT KTR AAA GAT TGT G-3', or 5'-CAR GCN AAA TAT KTR AAA GAT TGT G-3' (SEQ ID no. 2),
 - b) an oligonucleotide having the nucleotide sequence 5'-CAR GCA AAA TAT GTW AAG GAT TGT G-3' (SEQ ID no. 3),
 - 15 c) an oligonucleotide having the nucleotide sequence 5'-CAR GCW AAA ATT GTA AAR GAT TGT G-3' (SEQ ID no. 4),
 - d) an oligonucleotide having the nucleotide sequence 5'-CAA GCA AAA ATA GTA AAR GAC TGT G-3' (SEQ ID no. 5), and
 - e) an oligonucleotide having the nucleotide sequence 5'-CAR GCA
20 AAA TAT GTA AAA GAC TGT G-3' (SEQ ID no. 6),

wherein R = A or G, W = T or A, K = T or G, I = is inosine, and N = A, T, G, or C.

3. An oligonucleotide that may be used as a primer, in particular a reverse primer, to amplify a nucleic acid region of a genital human papilloma virus

having the nucleotide sequence 5'-ARY GGY TSY ARC CAA AAR TGR CT-3' (SEQ ID no. 7), wherein R = A, or G, Y = C, or T, and S = C, or G.

4. An oligonucleotide that may be used as a probe for detecting and/or for identifying genital HPV genotypes selected from the group comprising:

- 5 1) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 117 for detecting and/or identifying the HPV6 genotype,
- 2) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 118 for detecting and/or identifying the HPV11 genotype,
- 3) an oligonucleotide having the nucleotide sequence recited in SEQ
10 ID no. 19 for detecting and/or identifying the HPV16 genotype,
- 4) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 119 for detecting and/or identifying the HPV18 genotype,
- 5) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 120 for detecting and/or identifying the HPV31 genotype,
- 15 6) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 32 for detecting and/or identifying the HPV33 genotype,
- 7) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 41 for detecting and/or identifying the HPV35h genotype,
- 8) an oligonucleotide having the nucleotide sequence recited in SEQ
20 ID no. 44 for detecting and/or identifying the HPV39 genotype,
- 9) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 48 for detecting and/or identifying the HPV40 genotype,

- 10) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 121 for detecting and/or identifying the HPV42 genotype,
- 11) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 122 for detecting and/or identifying the HPV43 genotype,
- 5 12) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 123 for detecting and/or identifying the HPV44 genotype,
- 13) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 124 for detecting and/or identifying the HPV45 genotype,
- 10 14) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 125 for detecting and/or identifying the HPV51 genotype,
- 15) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 126 for detecting and/or identifying the HPV52 genotype,
- 16) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 127 for detecting and/or identifying the HPV53 genotype,
- 15 17) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 128 for detecting and/or identifying the HPV56 genotype,
- 18) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 82 for detecting and/or identifying the HPV58 genotype,
- 20 19) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 129 for detecting and/or identifying the HPV59 genotype,
- 20) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 130 for detecting and/or identifying the HPV66 genotype,

- 21) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 131 or 132 for detecting and/or identifying the HPV68 genotype,
- 22) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 133 for detecting and/or identifying the HPV70 genotype,
- 23) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 134 for detecting and/or identifying the HPV73 genotype, and
- 24) an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 135 for detecting and/or identifying the HPV82 genotype.
5. The oligonucleotide of one of Claims 1 to 4, that has a nucleotide sequence that is mutated relative to one of the nucleotide sequences recited in SEQ ID no. 1 to 7, 19, 32, 41, 44, 48, 82, or 117 to 135, obtained by the:
- a) deletion of 1 to 10 nucleotides in one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, 19, 32, 41, 44, 48, 82, or 117 to 135,
- b) addition of 1 to 10 nucleotides in one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, 19, 32, 41, 44, 48, 82, or 117 to 135, and/or
- c) substitution of 1 to 3 nucleotides in one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, 19, 32, 41, 44, 48, 82, or 117 to 135.
6. The oligonucleotide of Claim 5, wherein the deletion or addition of the nucleotides is present at the 5' end and/or 3' end of one of the nucleotide

sequences recited in SEQ ID nos. 1 to 7, 19, 32, 41, 44, 48, 82, or 117 to 135.

7. The oligonucleotide of one of Claims 1 to 6, wherein said oligonucleotide is a DNA molecule, RNA molecule, PNA molecule, LNA molecule, or a hybrid form thereof.

8. The oligonucleotide of one of Claims 1 to 7, wherein its nucleotide sequence is complementary to a sequence from the E1 gene region of at least one genital HPV genotype.

9. An oligonucleotide that has a nucleotide sequence that is complimentary over its entire length to the nucleotide sequence of an oligonucleotide of one of Claims 1 to 8.

10. A primer pair for amplifying a nucleic acid region of a genital human papilloma virus (HPV) comprising a forward primer and a reverse primer, wherein the forward primer is selected from the group comprising:

- a) an oligonucleotide of Claim 1 or 2 having one of the nucleotide sequences recited in SEQ ID nos. 1 to 6,
- b) an oligonucleotide of Claim 5 or 6, that has a nucleotide sequence that is mutated relative to the oligonucleotide of a), and
- c) a mixture of the oligonucleotides of a) and/or b),

and the reverse primer is selected from the group comprising:

- d) an oligonucleotide of Claim 3 having the nucleotide sequence recited in SEQ ID no. 7,
- e) an oligonucleotide of Claim 5 or 6, which has a nucleotide sequence that is mutated relative to the oligonucleotide of d), and

f) a mixture of the oligonucleotides of d) and e).

11. The primer pair of Claim 10, wherein the forward primer is an equimolar mixture of the oligonucleotides having the nucleotide sequences recited in SEQ ID nos. 2 to 6, and the reverse primer is the
5 oligonucleotide having the nucleotide sequence recited in SEQ ID no. 7.

12. A nucleic acid molecule comprising at least one region that has one of the nucleotide sequences recited in SEQ ID nos. 19, 32, 41, 44, 48, 82, or 117 to 135, a mutated nucleotide sequence thereof, obtained through the deletion and/or addition of 1 to 10 nucleotides and/or the substitution of 1
10 to 3 nucleotides in one of the nucleotide sequences recited in SEQ ID nos. 19, 32, 41, 44, 48, 82, or 117 to 135 or a complementary nucleotide sequence thereof and one or more additional regions having a total length of at least 1 nucleotide.

13. The nucleic acid molecule of Claim 12, wherein the additional regions
15 are not complementary to sequences of an amplification product that is obtained by means of an amplification process using a nucleic acid region of a genital human papilloma virus as the template and a primer pair of Claim 10 or 11.

14. The nucleic acid molecule of Claim 13, wherein the additional regions
20 have poly-A or poly-T nucleotide sequences.

15. The nucleic acid molecule of Claim 12, wherein the additional regions comprise multiple repetitions of a region that has one of the nucleotide sequences recited in SEQ ID nos. 19, 32, 41, 44, 48, 82, or 117 to 135, a mutated nucleotide sequence thereof, or a complementary nucleotide
25 sequence.

16. The nucleic acid molecule of Claim 15, wherein the repetitions are separated by spacers having a length of at least one nucleotide, or at least one phosphate, or at least one carbon atom, or at least one amino group.
17. A nucleic acid molecule that has a nucleotide sequence that, over its entire length, is complementary to the nucleotide sequence of a nucleic acid molecule of one of claims 12 to 16.
18. A nucleic acid molecule of one of Claims 12 to 17, wherein said molecule is a DNA molecule, RNA molecule, PNA molecule, LNA molecule, or a hybrid form thereof.
19. A process for amplifying a region of a nucleic acid of the genital human papilloma virus present in a biological sample, comprising the implementation of a nucleic acid amplification process using a primer pair comprising a forward primer and a reverse primer, wherein the forward primer is selected from the group comprising:
- a) an oligonucleotide of Claim 1 or 2 having one of the nucleotide sequences recited in SEQ ID nos. 1 to 6,
 - b) an oligonucleotide of Claim 5 or 6, having a nucleotide sequence that is mutated relative to the oligonucleotide of a), and
 - c) a mixture of the oligonucleotides of a) and/or b),
- and the reverse primer is selected from the group comprising:
- d) an oligonucleotide of Claim 3 having the oligonucleotide sequence recited in SEQ ID no. 7,
 - e) an oligonucleotide of Claim 5 or 6, having a nucleotide sequence that is mutated relative to the oligonucleotide of d), and

f) a mixture of the oligonucleotides of d) and e).

20. The process of Claim 19, wherein the oligonucleotide is a DNA molecule, RNA molecule, DNA molecule, LNA molecule, or a hybrid form thereof.

5 21. The process of Claim 19 or 20, wherein the biological sample is a smear of the cervix, a fresh tissue sample, a fixed tissue sample, or a cross-sectional specimen of a tissue sample.

22. The process of one of Claims 19 to 21, wherein the nucleic acid that is to be amplified is purified and/or isolated from the biological sample.

10 23. The process of Claim 22, wherein the nucleic acid to be amplified is a DNA.

24. The process of one of Claims 19 to 23, wherein the nucleic acid amplification process is a PCR (polymerase chain reaction) process.

15 25. The process of Claim 24, wherein the forward primer and the reverse primer in the nucleic acid amplification reaction are each used at a concentration of 0.5-1 pmoles/ μ L.

20 26. The process of Claim 25, wherein an equimolar mixture of the oligonucleotides having the nucleotide sequences recited in SEQ ID nos. 2 to 6 is used as the forward primer, wherein each oligonucleotide is present at a concentration of 0.1-0.2 pmoles/ μ L, and the oligonucleotide having the nucleotide sequence recited in SEQ ID no. 7 is used at a concentration of 0.5-1 pmoles/ μ L as the reverse primer.

27. The process of one of Claims 24 to 26, wherein the nucleic acid amplification is performed under the following temperature conditions:

25 a) heat to 95°C, with the temperature increased by 1°C per sec,

- b) hold the temperature at 95°C for 10 min,
- c) perform 40 cycles, each comprising 30 sec at 95°C, 30 sec at 55°C, and 1 min at 72°C,
- d) hold the temperature at 72°C for 5 min, and
- 5 e) cool to 4°C.

28. The process of one of Claims 19 to 23, wherein the nucleic acid amplification process is an LCR (ligase chain reaction) process, an NASBA process, or an isothermic process.

10 29. The process of one of Claims 19 to 28, wherein a region of the HPV gene E1 is amplified.

30. The process of one of Claims 19 to 29, wherein the amplified nucleic acid region is purified and/or isolated.

31. The process of one of Claims 19 to 30, wherein the amplification product is provided with a mark during or after the amplification reaction.

15 32. A process for detecting and/or identifying a genital HPV genotype, comprising the testing of a nucleic acid of a genital human papilloma virus present in a biological sample, in particular the testing of the HPV gene E1 or a portion thereof, by hybridization with at least one probe, wherein the probe is selected from the group comprising:

- 20 a) HPV genotype-specific oligonucleotides of Claim 4 having the nucleotide sequences recited in SEQ ID nos. 19, 32, 41, 44, 48, 82, or 117 to 135,

- b) the oligonucleotides of Claim 5 or 6, that have a nucleotide sequence that is mutated relative to one of the oligonucleotides of a),
 - 5 c) the oligonucleotides of Claim 9, that have a nucleotide sequence that is complementary to that of one of the oligonucleotides of a) or b),
 - d) nucleic acid molecules of one of Claims 12 to 18, and
 - e) mixtures of the oligonucleotides of a) to c) and/or of the nucleic acid molecules of d),
- 10 and the detection of the hybridization.

33. The process of Claim 32, wherein the oligonucleotide or nucleic acid molecule that is used as a probe is a DNA molecule, RNA molecule, PNA molecule, LNA molecule, or a hybrid form thereof.

15 34. The process of Claim 32 or 33, wherein the HPV nucleic acid that is present in the biological sample is amplified prior to hybridization with the probe.

35. The process of Claim 34, wherein the amplification of the nucleic acid is performed by means of a process of one of Claims 19 to 31.

20 36. The process of Claim 32 or 33, wherein the biological sample comprises an amplified nucleic acid region obtained by means of a process of one of Claims 19 to 31.

37. The process of one of Claims 32 to 36, wherein the biological sample is a smear of the cervix, a fresh tissue sample, a fixed tissue sample, or a cross-sectional specimen of a tissue sample.

38. The process of one of Claims 19 to 37, wherein the process is used for the diagnosis and/or early detection of diseases, precursor stages of diseases, risks of diseases, and/or pathological changes caused by genital human papilloma viruses.
- 5 39. The process of Claim 38, wherein the disease is a cancer disease.
40. A nucleotide array for detecting and/or identifying the genotype of a human papilloma virus contained in a biological sample, in particular using a process of one of Claims 32 to 39, comprising a solid carrier having a surface and at least one first oligonucleotide or nucleic acid molecule
- 10 bound to the carrier surface, which is suitable for detecting and/or identifying a genital HPV genotype selected from the group comprising:
- a) HPV genotype-specific oligonucleotides of Claim 4 having the nucleotide sequences recited in SEQ ID nos. 19, 32, 41, 44, 48, 82, or 117 to 135,
 - 15 b) oligonucleotides of Claim 5 or 6 that have a nucleotide sequence that is mutated relative to one of the oligonucleotides of a),
 - c) oligonucleotides of Claim 9 that have a nucleotide sequence that is complementary to one of the oligonucleotides of a) or b),
 - d) nucleic acid molecules of one of Claims 12 to 18, and
 - 20 e) mixtures of the oligonucleotides of a) to c) and/or of the nucleic acid molecules of d).
41. The nucleotide array of Claim 40, wherein the carrier is platelet-shaped, for example in the form of a microscope slide, or is platelet-shaped with depressions, as for example a chamber slide, or such as a

microtiter plate having the dimensions stated in the recommendations of the SBS (Society of Biomolecular Screening).

42. The nucleotide array of Claim 40, or 41, wherein the first oligonucleotides or nucleic acid molecules on the surface of the carrier are
5 located in a defined analysis area.

43. The nucleotide array of one of Claims 40 to 42, wherein the surface of the carrier has a control area.

44. The nucleotide array of Claim 43, wherein the control area comprises a control for orienting the carrier, an amplification control, a hybridization
10 control, a sample control, and/or a print control.

45. The nucleotide array of Claim 44, wherein the control for orienting the carrier comprises at least one second oligonucleotide or nucleic acid molecule.

46. The nucleotide array of Claim 45, wherein the second oligonucleotide
15 is a fluorescent oligonucleotide, and the control for orienting the carrier comprises at least three spots of the fluorescent oligonucleotide.

47. The nucleotide array of Claim 44, wherein the amplification control comprises at least one third oligonucleotide or nucleic acid molecule.

48. The nucleotide array of Claim 47, wherein the third oligonucleotide or
20 nucleic acid molecule is suitable for use as a probe for detecting an amplification product that is obtained by means of an amplification process using a control nucleic acid as the template and a primer pair of Claim 10 or 11.

49. The nucleotide array of Claim 48, wherein the control nucleic acid has
25 a length and a GC content that corresponds to the length and the GC

content of the amplification product that is obtained by means of an amplification process using the nucleic acid region of a genital human papilloma virus as the template and a primer pair of Claim 10 or 11.

50. The nucleotide array of Claim 44, wherein the hybridization control
5 comprises at least one fourth oligonucleotide or nucleic acid molecule.

51. The nucleotide array of Claim 50, wherein the hybridization control comprises at least two to 10 spots of the fourth oligonucleotide or nucleic acid molecule, and the spots have variously defined amounts of the fourth oligonucleotide or nucleic acid molecule.

10 52. The nucleotide array of Claim 51, wherein the hybridization control comprises spots with a dilution series of the fourth oligonucleotide or nucleic acid molecule.

53. The nucleotide array of Claim 44, wherein the sample control comprises at least one fifth oligonucleotide or nucleic acid molecule.

15 54. The nucleotide array of Claim 53, wherein the fifth oligonucleotide or nucleic acid molecule is suitable for use as a probe for detecting the human ADAT1 gene.

55. The nucleotide array of Claim 44, wherein the print control comprises at least one sixth oligonucleotide or nucleic acid molecule.

20 56. Nucleotide array of one of Claims 40 to 55, wherein the oligonucleotides and nucleic acid molecules are embodied as DNA molecules, RNA molecules, PNA molecules, LNA molecules, or hybrid forms thereof.

57. A kit for detecting and/or identifying genital HPV genotypes,
25 comprising at least one first container having at least one primer for

amplifying regions of the HPV gene E1, selected from oligonucleotides of one of Claims 1 to 3, oligonucleotides of Claim 5 or 6 having a nucleotide sequence that is mutated relative to one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, and/or primer pairs of Claim 10 or 11, and at least one second container having at least one probe for detecting an amplified region of the HPV gene E1, selected from oligonucleotides of one of Claims 4 to 9, that has one of the nucleotide sequences recited in SEQ ID nos. 19, 32, 41, 44, 48, 82, or 117 to 135, a mutated sequence thereof, or a complementary sequence thereof, and nucleic acid molecules of one of Claims 12 to 18.

58. The kit of Claim 57, comprising at least 24 second containers having at least 24 different probes for detecting and/or identifying the HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV35h, HPV39, HPV40, HPV42, HPV43, HPV44, HPV45, HPV51, HPV52, HPV53, HPV56, HPV58, HPV59, HPV66, HPV68, HPV70, HPV73, and HPV82 genotypes, wherein each container contains at least one probe and wherein all probes contained in a container can detect only one specific genital HPV genotype.

59. A kit for detecting and/or identifying genital HPV genotypes, comprising at least one first container having at least one primer for amplifying regions of the HPV gene E1, selected from oligonucleotides of one of Claims 1 to 3, oligonucleotides of Claim 5 or 6 having a nucleotide sequence that is mutated relative to one of the nucleotide sequences recited in SEQ ID no. 1 to 7, and/or primer pairs of Claim 10 or 11, and the nucleotide array of one of Claims 40 to 56.

60. A kit of one of Claims 57 to 59, comprising two first containers, wherein one container contains equimolar amounts of the oligonucleotides having the nucleotide sequences recited in SEQ ID nos. 2 to 6, or mutated sequences thereof, and one container contains an oligonucleotide having

the nucleotide sequence recited in SEQ ID no. 7 or a mutated nucleotide sequence thereof.

61. A kit of one of Claims 57 to 59, comprising six first containers, wherein five containers each contain one of the oligonucleotides having the
5 nucleotide sequences recited in SEQ ID nos. 2 to 6, or mutated sequences thereof, and one container contains an oligonucleotide having the nucleotide sequence recited in SEQ ID no. 7, or a mutated nucleotide sequence thereof.

62. A kit of one of Claims 57 to 61, additionally comprising a container
10 having a control nucleic acid that may be amplified using an oligonucleotide of Claim 1 or 2 with one of the nucleotide sequences recited in SEQ ID nos. 1 to 6 as the forward primer, and a nucleotide of Claim 3 having the nucleotide sequence recited in SEQ ID no. 7 as the reverse primer.

15 63. The use of an oligonucleotide of Claim 4, of an oligonucleotide of Claim 5 or 6, whose nucleotide sequence is mutated relative to one of the nucleotide sequences recited in SEQ ID nos. 19, 32, 41, 44, 48, 82, or 117 to 135, of an oligonucleotide of Claim 9, whose nucleotide sequence is complementary to one of the nucleotide sequences recited in SEQ ID nos.
20 19, 32, 41, 44, 48, 82, or 117 to 135, or of a mutated nucleotide sequence thereof, or of a nucleic acid molecule of one of Claims 12 to 18 for detecting and/or identifying a genital HPV genotype.

64. The use of an oligonucleotide of one of Claims 1 to 3, of an oligonucleotide of Claim 5 or 6, whose nucleotide sequence is mutated
25 relative to one of the nucleotide sequences recited in SEQ ID nos. 1 to 7, or of a primer pair of Claim 10 or 11 for amplifying a nucleic acid region of a genital human papilloma virus.

65. The use of an oligonucleotide of one of Claims 1 to 9, of a nucleic acid molecule of one of Claims 12 to 18, or of a primer pair of Claim 10 or 11 for the diagnosis and/or early detection of diseases caused by genital human papilloma viruses.
- 5 66. The use of an oligonucleotide of one of Claims 1 to 9, of a nucleic acid molecule of one of Claims 12 to 18, or of a primer pair of Claim 10 or 11 to produce a means for diagnosing diseases caused by genital human papilloma viruses.
- 10 67. The use of Claim 66, wherein the means is a nucleotide array of one of Claims 40 to 56.
68. The use Claim 66, wherein the diagnostic means is a kit of one of Claims 57 to 62.
- 15 69. The use of one of Claims 63 to 68, wherein the oligonucleotide or nucleic acid molecule is a DNA molecule, RNA molecule, PNA molecule, LNA molecule, or a hybrid form thereof.

Summary

The present invention relates to oligonucleotides that are suitable for use as primers for amplifying the DNA of genital human papilloma viruses (HPV), oligonucleotides that are suitable for use as probes for typing
5 genital HPV genotypes, processes for the amplification of the DNA of genital human papilloma viruses, processes for detecting and/or identifying genital HPV genotypes, nucleotide microarrays and kits comprising said oligonucleotides, as well as the use of the oligonucleotides for amplifying or typing genital HPV genotypes, for the
10 diagnosis and/or early detection of diseases, as well as for preparing means for diagnosing diseases.